constitutively active Rho GTPases demonstrated essentially cytoplasmic localization. Moreover, active Cdc42 was shown to augment localization of mDia2 to cell plasma membrane and slow down its nuclear shuttling. Finally, we have found that even without LMB treatment, GFP-fused full length mDia2 is accumulated to the nuclear rim, where it colocalizes with nesprin, a protein involved in linking nucleus with actin cytoskeleton. Constitutively active Rac1 was also enriched in the nuclear rim. The experiments using cell treatment with digitonin, which permeabilizes plasma- but not the nuclear membrane, suggested that mDia2-enriched nuclear rim was located at the cytoplasmic side of the nuclear membrane.

1913-Pos Board B683
Non-Muscle Myosin IIA at the Immunological Synapse: Integration of Mechanical Regulation with T Cell Signaling
Yan Yu[1], Nicole C. Fay[2], Alexander A. Smoligovets[2], Hung-Jen Wu[1], Jay T. Groves[1,3].
[1]Department of Chemistry, Univ of California-Berkeley, Berkeley, CA, USA, [2]Department of Molecular and Cell Biology, Univ of California-Berkeley, Berkeley, CA, USA, [3]Howard Hughes Medical Institute, Berkeley, CA, USA.
Activation of T cell receptor (TCR) by antigens occurs in concert with an elaborate multi-scale spatial reorganization of proteins at the immunological synapse, the junction between a T cell and an antigen-presenting cell (APC). Signaling through discrete T cell receptors (TCRs) in the context of immunological synapse, involves the orchestrated movement and reorganization of TCR microclusters. The frictional coupling between the actin cytoskeleton and protein microclusters has been proposed to be essential for the spatial organization of signaling receptors at all length scales, but the role of molecular motors in this process is largely unknown. By using the hybrid live T cells-supported membrane platform, we study the role of myosin motors in the synapse formation of primary T cells and explore a possible mechanical mechanism of signal modulation involving myosin II.

1914-Pos Board B684
Three Dimensional Single Molecule Tracking of Full Length Myosin Conformation Change
Janet Y. Sheung[1], Kathleen M. Trybus[2], Paul R. Selvin[1].
[1]University of Illinois at Urbana-Champaign Physics Department and Center for the Physics of the Living Cells, Urbana, IL, USA, [2]University of Vermont Department of Molecular Physiology & Biophysics, Burlington, VT, USA.
Myosin Va is a motor protein responsible for vesicular transport inside eukaryotic cells. Its structure has been well-studied and is known to contain a flexible hinge region approximately half-way between the N-terminal motor domain and C-terminal globular tail. Previous studies have shown two distinct conformations for Myosin Va, one where the hinge remains extended and the protein is active, and one where the hinge allows the protein to fold, allowing auto-inhibition between the globular tail and motor regions. It is assumed, but never shown at the single-molecule level, that Myosin Va actively switches between these conformations at the timescales typical of their runs. Traditional two-dimensional fluorescence tracking techniques cannot adequately capture the conformation change. However, it is possible to track an out-of-focus particle in all three dimensions with a modified form of FIONA microscopy. The point spread function of the out-of-focus particle takes the shape of an Airy disc, with the width of the disc proportional to the distance by which the particle is out of focus. With this technique, the spatial resolution degrades quickly with drift, so we have further modified the microscope with an auto-focus system based on feedback of backscattered signal from a secondary IR laser. We present tracking data of quantum dots bound to full-length Myosin Va with < 5 nm spatial resolution in XY, < 30 nm in Z at a time resolution of 100 ms, which is sufficient to resolve conformation changes.

Intracellular Cargo Transport

1915-Pos Board B685
Testing the Force Response of the Allosteric Cycle of Myosin VI using Brownian Dynamics
Riina Tehver, Andrew McCallister.
Denison University, Granville, OH, USA.
A central question in the study of many processive motor proteins is how are the mechano-chemical cycles in the two motor domains coordinated. In our study, we investigate the hypothesis that the coordination is achieved via subtle changes in the reaction rates due to asymmetric tension within the structures. We perform coarse-grained Brownian dynamics simulations of the myosin VI motor domain and study structural changes in the nucleotide binding pocket as a function of applied tension and relate the structural changes to the changes in the chemical reaction rates.

1916-Pos Board B686
Modeling the Endosomal Escape of Labeled Cell Penetrating Peptides
Fatemeh Madani.
Biochemistry and Biophysics- Stockholm University, Stockholm, Sweden.
Table of Contents

1917-Pos Board B687
Diffusion inside Living Human Cells
Natascha Leijnse[1], Jae-Hyung Jeon[2], Steffen Loft[3], Ralf Metzler[4,2], Lene B. Oddershede[1].
[1]Niels Bohr Institute, University of Copenhagen, Copenhagen, Denmark, [2]Department of Physics, Tampere University of Technology, FI-33101 Tampere, Finland, [3]Institute for Public Health, University of Copenhagen, Copenhagen, Denmark, [4]Physics Department T30g, Technical University of Munich, Munich, Germany.
Naturally occurring lipid granules diffuse in the cytoplasm and can be used as tracers to map out the viscoelastic landscape inside living cells. Using optical trapping and single particle tracking we found that lipid granules exhibit anomalous diffusion inside human umbilical vein endothelial cells. For these cells the exact diffusional pattern of a particular granule depends on the physiological state of the cell and on the localization of the granule within the cytoplasm. Granules located close to the actin rich periphery of the cell move less than those located towards to the center of the cell or within the nucleus. Also, granules in cells which are stressed or have attached to a surface for a long period of time move in a more restricted fashion than within healthy cells. For granules diffusing in healthy cells, in regions away from the cell periphery, occurrences of weak ergodicity breaking are observed, similar to the recent observations inside living fission yeast cells [1].
[1] J.-H. Jeon, V. Tejedor, S. Burov, E. Barkai, C. Selhuber-Unkel, K. Berg-Sørensen, L. B. Oddershede, and R. Metzler, Phys. Rev. Lett. 106, 048103 (2011).

1918-Pos Board B688
Dissecting Molecular Motor Models
Neville J. Boon, Rebecca B. Hoyle.
University of Surrey, Guildford, United Kingdom.
A molecular motor is a nano-scale protein which converts chemical energy into mechanical work. For example, myosin-V is a double headed processive molecular motor that transports a variety of cargos within biological cells. It achieves this by walking head-over-head along an actin track, passing through a sequence of coordinated chemical reactions and mechanical motions, taking several successive steps before detaching.
There is much debate as to the exact nature of the stepping mechanism of myosin-V due to the noise to which nano-scale measurements are subject. Mechanochemical aspects have been experimentally investigated and averaged quantities, such as velocities and run lengths, have been measured. Our work focuses on theoretical methods to extract more information - such as a more precise stepping mechanism - from these experimental results aiming to improve the quality of molecular motor modelling.
A postulated sequence of mechanochemical changes a molecule undergoes can be encoded into a discrete stochastic model. Optimisations techniques are used to fit parameters of the system against measureable quantities, giving a quantitative measure as to the degree to which the system agrees with experiment.
The validity of several models for myosin-V is discussed and ideas such as the detachment mechanism of the protein are critically analysed. Existing theoretical techniques are tested and improved. A quantitative analysis of competing stepping cycles and their agreement with experimental results is also discussed.

1919-Pos Board B689
Illuminating the Intraflagellar Transport Machinery of Caenorhabditis Elegans
Bram Prevo[1], Pierre J.J. Mangeol[1], Jonathan M. Scholey[2], Erwin J.G. Peterman[1].
[1]VU University Amsterdam, Amsterdam, Netherlands, [2]University of California, Davis, Davis, CA, USA.
Molecular motors of the kinesin and dynein superfamilies are the driving force behind intracellular transport, cell division and cell propulsion. Inside the